Direct Spectrophotometric Observation of an *O*-Acylisourea Intermediate: Concerted General Acid Catalysis in the Reaction of Acetate Ion with a Water-soluble Carbodi-imide [1a]

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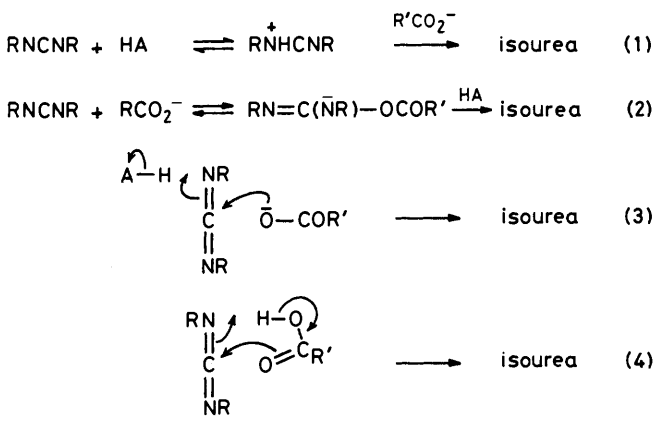
The rate constants for formation and decay of *O*-acylureas from carbodi-imide and acids have been measured using aqueous media. The *O*-acetylisourea from acetate and *N*-ethyl-*N'*-(3-trimethylammoniopropyl)carbodi-imide (ETC) possesses an acidic group of p*K* 6.8 and decomposes (k_2) in its acid form as the dication by reaction with acetate ion or water. The reaction of the carboxylate anion with ETC is general acid catalysed ($k_1 = \Sigma k_{HA}[RCO_2^-][HA]$) and the deuterium oxide solvent isotope effect indicates a rate-limiting proton transfer except for the oxonium ion acting as acid. The Brønsted α value for variation of the structure of HA (0.67) is consistent with a proton transfer concerted with nucleophilic attack by the acetate anion. A concerted mechanism is consistent with the weak basicity of the carbodi-imide and the weak acidity of the isourea adduct. The third-order term involving acetic acid, acetate ion, and carbodi-imide carries *ca.* 60% of the total reaction flux at pH 6.80 and 1M total acetic acid buffer concentration. At this pH *ca.* 40% of the reaction flux goes through the stepwise ' Khorana ' mechanism with specific acid catalysis. Intramolecular general acid catalysis is demonstrated to occur in the reaction of 2,2-diethylmalonic acid monoanion with ETC and the effective molarity compared with intermolecular catalysis is 15M. Attack of carboxylate anions on ETC with *N*-chloroethylmorpholinium ion as the general acid has a Brønsted type β_N of 0.46.

Khorana [1b] proposed that the formation of *O*-acylisourea in peptide synthesis involves protonation of the carbodi-imide followed by rate-limiting attack of the carboxylate ion [equation (1)]. The proton from the carboxy-group may be transferred through a number of other routes [equations (2)—(4)]. Proton transfer may occur after attack of carboxylate ion on the carbodi-imide [equation (2)] brought about by a general acid. The reaction may occur through a concerted mechanism [equation (3)] where a general acid donates a proton as the carboxylate ion attacks; an alternative concerted reaction [equation (4)] involves intramolecular proton transfer from the attacking carboxylic acid.

Few kinetic mechanistic studies have been carried out on the addition of carboxylic acids to carbodi-imides.[2] Alkyl- and aryl-carbodi-imides suffer from the problem of water insolubility and in order to obtained reproducible and easily interpreted kinetic data it is preferable to carry out reactions with a significant proportion of water.[3] We are interested in the possibility of buffer catalysis and if observations of rate-limiting proton transfer are made for aqueous solution then this will be significant in the non-aqueous case; the inverse argument does not hold. We choose as our standard carbodi-imide the water-soluble *N*-ethyl-*N'*-(3-trimethylammoniopropyl)carbodi-imide (ETC) perchlorate; previous studies [4] indicated that we could easily follow the kinetics of reaction of carbodi-imides using a standard assay [4] or through a change in u.v. spectrum.[5]



Experimental

Materials.—Commercially available carboxylic acids were obtained as the free acid or as the potassium salt and were purified by distillation or recrystallisation.

Trimethylamine, *N*-methylmorpholine, and *N*-chloroethylmorpholine were obtained from Aldrich and converted into their hydrochlorides by bubbling HCl gas into their ethereal solutions; the HCl salts were recrystallised from ethanol. *N*-Propargylmorpholine [6] was prepared by adding propargyl bromide (70 g, 0.6 mol) to morpholine (140 g, 1.2 mol) in anhydrous ether (300 ml). The mixture was stirred and refluxed for 16 h; the resulting suspension was filtered and the filtrate evaporated. The residue was distilled to yield *N*-propargylmorpholine (73%), b.p. 184—186° at 760 Torr. *NN*-Dimethylaminoacetonitrile [7] was prepared by adding aqueous dimethylamine solution (26.4 g of 25% stock, 0.16 mol) to an ice-cooled solution of aqueous formaldehyde (13.2 g of 37% stock, 0.16 mol) at a rate to ensure that the temperature did not exceed 25°. Sodium cyanide (7.9 g, 0.15 mol) was added and after 1 h concentrated HCl (12.2 ml, 0.14 mol) was added slowly with stirring. The solution was allowed to stand overnight and then extracted with chloroform; the dried chloroform was evaporated to give the free nitrile. The HCl salt of *NN*-dimethylaminoacetonitrile was prepared from concentrated HCl and recrystallised from HCl–acetone as needles, m.p. 149—150° (lit.,[7] 150—151.5°).

N-Ethyl-*N'*-(3-trimethylammoniopropyl)carbodi-imide (ETC) was prepared as its iodide salt and converted to a solution of the perchlorate for kinetic studies as previously described.[5]

N-*Phenethyl*-N'-(3-*dimethylaminopropyl*)*urea* was prepared from phenethyl isocyanate and 3-dimethylaminopropylamine; it had m.p. 82—83° (Found: C, 67.3; H, 9.4; N, 17.0. $C_{14}H_{23}N_3O$ requires C, 67.5; H, 9.2; N, 16.9%).

N-*Phenethyl*-N′-(3-*trimethylammoniopropyl*)*urea toluene*-4-*sulphonate* was prepared from the dimethylamino-compound with methyl toluene-4-sulphonate; it had m.p. 120—121° (Found: C, 60.5; H, 7.7; N, 9.3. $C_{22}H_{33}N_3O_4S$ requires C, 60.7; H, 7.6; N, 9.7%).

N-*Phenethyl*-N′-(3-*dimethylaminopropyl*)*carbodi-imide* was prepared from the urea using toluene-4-sulphonyl chloride and triethylamine. It was an oil, b.p. 134° at 0.45 Torr (Found: C, 72.6; H, 8.8; N, 17.7. $C_{14}H_{21}N_3$ requires C, 72.7; H, 9.1; N, 18.2%).

N-*Phenethyl*-N′-(3-*trimethylammoniopropyl*)*carbodi-imide toluene*-4-*sulphonate* was prepared from the parent carbodi-imide by treating it with methyl toluene-4-sulphonate. It had m.p. 106—107° (Found: C, 63.2; H, 7.8; N, 10.1. $C_{22}H_{31}N_3O_3S$ requires C, 63.3; H, 7.4; N, 10.1%).

N-*Ethyl*-N′-(3-*phenethyldimethylammoniopropyl*)*urea toluene*-4-*sulphonate* was prepared by treating *N*-ethyl-*N*′-(3-dimethylaminopropyl)urea with phenethyl toluene-4-sulphonate. It had m.p. 145—146° (Found: C, 61.3; H, 8.0; N, 9.3. $C_{23}H_{35}N_3O_4S$ requires C, 61.5; H, 7.8; N, 9.4%).

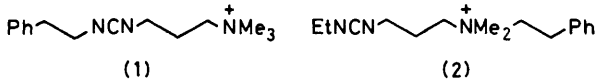
The preparation of the *N*-ethyl-*N*′-(3-phenethyldimethylammoniopropyl)carbodi-imide toluene-4-sulphonate salt was not possible in our hands. We tried reacting the toluenesulphonate with the carbodi-imide under a variety of conditions but only obtained oils; it is likely that the oils were the required product and that some phase effect was preventing formation of crystals. In any case the material was not able to be sufficiently purified to enable it to be used in product analysis studies.

All the substrates and buffer reagents prepared for this study had satisfactory i.r. and n.m.r. spectra.

Deuterium oxide (99.7 atom %D) was obtained from Merck, Sharp and Dohme Ltd. *N*-Methylmorpholine DCl salt was prepared by dissolving *N*-methylmorpholine HCl in D_2O and evaporating the solvent under reduced pressure. The remaining salt was treated three times *via* the same procedure to ensure complete exchange. Water, used throughout the investigation, was distilled twice from glass. Other buffers and reagents were of analytical quality or were recrystallised or redistilled from bench grade materials.

Methods.—The kinetics of the reaction of carboxylic acids with ETC were measured spectrophotometrically at an appropriate wavelength. A typical experiment involved adding a solution of the carbodi-imide (0.05 ml) on the flattened tip of a glass rod to a buffer solution (2.5 ml) in a silica cell in the thermostatted cell holder of a Unicam SP 500 spectrophotometer. Two or three rapid vertical strokes of the glass rod in the solution effected complete stirring and the absorbance at a fixed wavelength was recorded on an external Servoscribe recorder. The pH of the solution in the cell was measured after the reaction using a Radiometer pH-meter PHM26 calibrated with E.I.L. buffers to ±0.01 units. Stock solutions of the carbodi-imide perchlorate in water were at *ca.* 0.4M and were stored for no longer than a day. The above spectrophotometric procedure was also adopted to determine the optimum wavelength for study using an SP 800 wavelength scanning instrument. The traces of absorbance were analysed by plotting $A_t - A_\infty$ *versus* time on two cycle semi-logarithmic graph paper. Reactions obeying first-order rate laws gave linear plots.

The reaction of carbodi-imide with acetic acid eventually yields urea and acetic acid; when an O ⟶ N acyl migration occurs the yield of acetic acid will be <100% and this reaction may therefore be detected by pH titration. The ETC is added to the acetic acid buffer at pH values between 4 and 7 to give a final composition of 0.1M-acetate in 5 ml at 25°; the addition takes *ca.* ¼ h and the solution is kept for ½ h to ensure complete reaction. The pH is then adjusted to 9 and the solution titrated with HCl (4M). Adding the ETC to the acetate buffer ensures that the preparative experiment has the conditions of the kinetic experiments as close as is possible, namely the acetate in large excess over the ETC. The amount of acetic acid in the product is taken from the titration curve and compared with a control experiment carried out without carbodi-imide.



(1) (2)

It is difficult to assay for products in the reaction of ETC owing to the presence of polar side chains; moreover the absence of a significant u.v. spectrum of the products handicaps the product analysis. We therefore synthesised a carbodi-imide analogous to ETC but containing a chromophore suitable for detection by u.v. from a high pressure liquid chromatography (h.p.l.c.) column. We investigated two possible derivatives (1) and (2) where the chromophore will not alter significantly the mechanism or reactivity. The synthesis of (2) proved difficult and (1) was finally successful. We also synthesised the corresponding urea to calibrate the chromatography column. Treatment of (1) with acetic acid buffers was carried out under the conditions of the above experiments. Analysis of the product by h.p.l.c. showed only one species namely the urea; the toluenesulphonic acid was eluted on the solvent front. The h.p.l.c. apparatus was a Pye-Unicam instrument comprising an LC-XPD pump, LC-UV detector, LC-XP dialamix, and PM-8251 single pen recorder. The column (25 cm × 4.5 mm) was packed with Lichrosorb RP 8 reverse phase packing; we are grateful to Mrs. J. A. Holland of Shell Research Ltd., Sittingbourne for packing the column. The eluant was a 40% methanol–water solution; the aqueous component was prepared by dissolving n-pentanesulphonic acid (0.005M) in water containing 3 ml acetic acid per litre.

Buffering of the reaction media at pH values outside the capacity of acetic acid–acetate buffer was effected using a machine described previously which delivers acid or base to the stirred reaction mixture in the 2.5 ml silica cuvette in the cell compartment of the spectrophotometer.[8] Potentiometric titrations were carried out using a Radiometer titration set comprising REC 61 Servograph, REA titratigraph, PHM 26 pH-meter, TTT 60 titrator, and ABU 11 autoburette.

Spectrophotometric measurements of p*K* were carried out using a Unicam SP 800 instrument. The substrate was dissolved in 1M-KCl solution (2.5 ml) in a silica cell in the thermostatted cell compartment of the spectrophotometer. The cell solution was stirred magnetically by the machine described above [8] and acid or base added from the pH-stat apparatus to adjust the pH to a given value; the absorption at an appropriate wavelength was measured. The process was continued until a pH-profile of the absorption was obtained; the ionisation constant was determined from this pH-dependence using the Henderson–Hasselbach equation. The time required for such a measurement is very short compared with that using buffers. Fitting the experimental data to theoretical equations was accomplished using ' Basic Language ' programs; we acknowledge the help of Dr. C. R. Farrar.

Results

Analysis by the potentiometric method indicates that no overall consumption of acetic acid occurs when ETC is added to acetate buffers over the pH range 4—7. The reaction carried out over the same pH range with the chromophoric *N*-phenethyl-*N*′-(3-trimethylammoniopropyl)carbodi-imide (1)

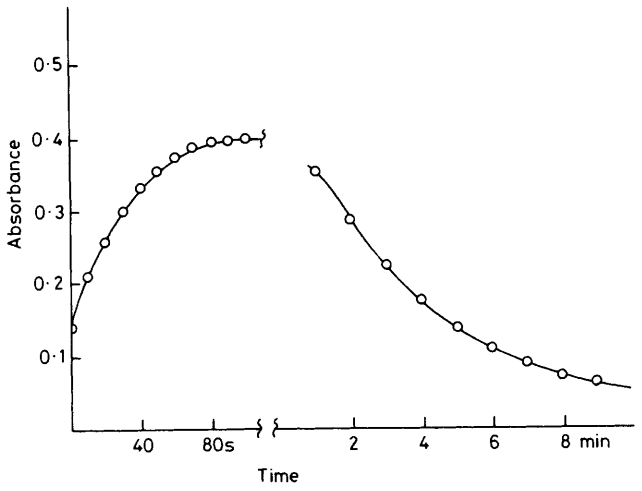


Figure 1. Time course for the absorbance (250 nm) for the reaction of ETC at 0.004M in 1M-NaOAc at pH 6.86; the base line represents the absorbance at infinite time; the absorbance has been backed-off by 0.4 units and the line is theoretical from data for k_1 and k_2

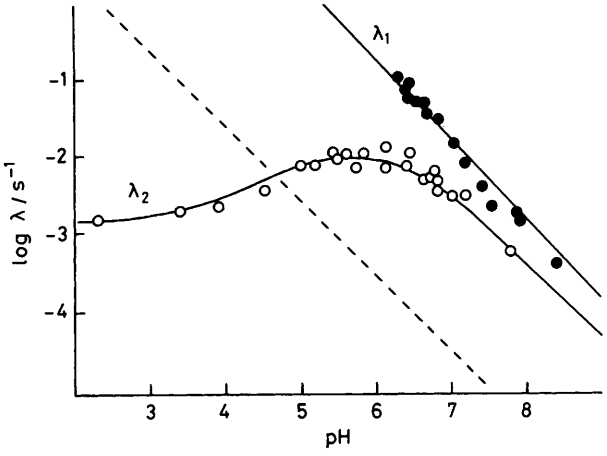


Figure 2. Dependence on pH of the reaction of ETC with aqueous acetic acid buffer at 1M total concentration, 25°, and 1M ionic strength. The lines are theoretical from the equations in the text and the dashed line is theoretical for the hydrolysis of ETC in the absence of buffers [5]

reveals only the urea on h.p.l.c. analysis and no peaks which could correspond with the rearranged isourea; comparison of peak areas with that of the standard *N*-phenethyl-*N'*-(3-trimethylammoniopropyl)urea indicates that a theoretical amount of urea is produced. In both experiments carbodi-imide is added slowly to the acetate buffer so that it is never in excess in order to duplicate the conditions of the kinetic experiments where acetate is always in large excess over carbodi-imide.

The reaction of ETC in aqueous acetate buffers is illustrated in Figure 1 and reveals the presence of an intermediate species. The kinetics are described by the first-order time constants λ_1 and λ_2 which relate to the mechanism of equation (5). In all the cases studied $\lambda_1 \gg \lambda_2$ so that complicated methods for measuring these time constants are not necessary. Analysis of

$$\text{Acetate} + \text{ETC} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Intermediate} \xrightarrow[\text{AcO}^-]{k_2} \text{Product} \qquad (5)$$

the mechanism of equation (5) leads to the kinetic expression (6) where the components are defined by equations (7) and (8). Combining equations (7) and (8) leads to $\lambda_1 + \lambda_2 = k_1 + k_{-1} + k_2$ and $\lambda_1 . \lambda_2 = k_1 . k_2$. Since the maximal absorbance

$$\text{Intermediate} = B_1 e^{-\lambda_1 t} + B_2 e^{-\lambda_2 t} \qquad (6)$$

$$\lambda_1 = \tfrac{1}{2}[(k_{-1} + k_1 + k_2) + \sqrt{(k_{-1} + k_1 + k_2)^2 - 4k_1k_2}] \qquad (7)$$

$$\lambda_2 = \tfrac{1}{2}[(k_{-1} + k_1 + k_2) - \sqrt{(k_{-1} + k_1 + k_2)^2 - 4k_1k_2}] \qquad (8)$$

reached in the experiments is independent of pH and concentration of acetate buffer (down to 0.2M) $k_{-1} \ll k_1$; since $\lambda_1 \gg \lambda_2$ $\lambda_1 = k_1 + k_2$ and hence $k_1 = \lambda_1$ and $k_2 = \lambda_2$.

The O ⟶ N acyl transfer by-pass mechanism is not significant in the reaction studied here and is neglected.

Decay Rate Constant k_2.—The rate constant k_2 is proportional to the concentration of acetate ion in the pH region 5—8. At pH 2.33 no dependence on total acetate concentration is observed and over a pH range from 2.3 to 8 at 1M total acetate buffer concentration the time constant varies as shown in Figure 2. The rate law governing these results is equation (9) where k_w, k_{AcO^-}, and K are respectively 1.8×10^{-3} s^{-1}, 7.9×10^{-3} l mol^{-1} s^{-1} and $10^{-6.8}$.

$$k_2 = (k_w + k_{AcO}[AcO^-])/(1 + K/a_H) \qquad (9)$$

The possibility that the observed intermediate is acetic anhydride (as is observed by Knorre and his co-workers [2d] for a similar water-soluble carbodi-imide under different conditions) is excluded for this study by the observation of Butler and Gold [9] that the hydrolysis of acetic anhydride is catalysed by acetate buffers according to the kinetic law 10^3k (s^{-1}) = 2.42 + 4[AcO^-] as opposed to the law obtained for the intermediate [equation (9)]. Knorre and his co-workers [2d] observed a pH-*independent* rate constant for degradation of the acetic anhydride intermediate from pH 1.5 to 7; this result is expected as there was essentially no acetic buffer present, it having been depleted in the formation of the intermediate from the carbodi-imide. Knorre's experiments are complicated by the concentration of the reactants (both at 0.1M) so that during the hydrolysis of the acetic anhydride intermediate an appreciable build up of acetate will occur presumably catalysing the hydrolysis.[9] The rate constants for acetic anhydride decomposition are larger than those for the intermediate observed here especially in the alkaline region and we propose that, although acetic anhydride is formed as a result of acetate attack on the intermediate it is degraded in a fast step.

Formation Rate Constant k_1.—The formation rate constant (k_1) depends on the square of the total acetate buffer concentration. Figure 3 illustrates this dependence for a range of pH values and the rate law governing k_1 is given in equation (10); the parameter k_H and k_{HOAc} were derived from plots of k_1/[total acetic acid] *versus* [total acetic acid] concentration. Intercepts and slopes of this plot are proportional to FA and

$$k_1 = k_H a_H[AcO^-] + k_{HOAc}[HOAc][AcO^-] \qquad (10)$$

FA(1 − FA) respectively as in equation (11) and as illustrated

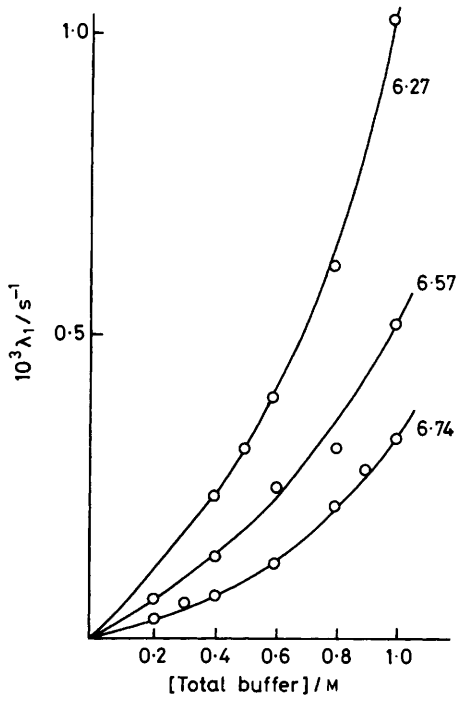


Figure 3. Representative plots of k_1 *versus* total acetate buffer concentration for reaction of ETC at 25° and 1M ionic strength. Numbers refer to the pH values of the individual buffers and the lines are theoretical from equation (10) and parameters from Table 1

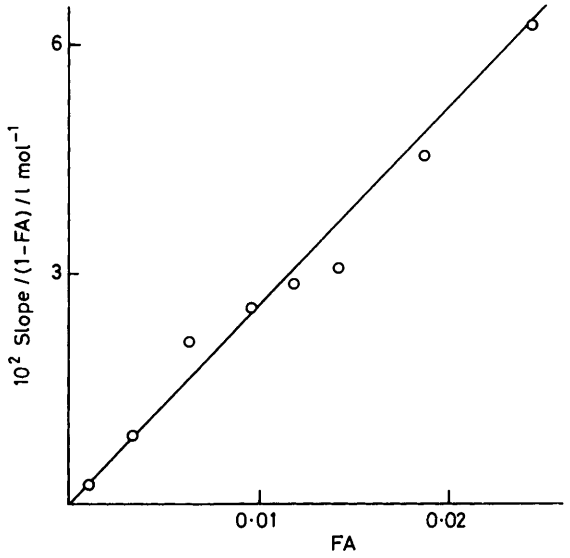


Figure 4. Plot of the slope/(1 < FA) *versus* FA for the reaction of acetate buffers with ETC derived from the plot of k_1/total acetate concentration against total acetate concentration. The line is theoretical from parameters in Table 1 and equation (11)

$$k_1/[\text{total acetic acid}] = k_H K_a FA + k_{HOAc} FA(1 - FA)[\text{total acetic acid}] \quad (11)$$

in Figures 4 and 5. The term FA is the fraction of total acetate buffer present as the free acid and K_a is the ionisation constant of acetic acid. The gradient plotted in Figure 4 is $k_{HOAc}FA(1 - FA)$ and the intercept plotted as the ordinate in Figure 5 is $k_H K_a FA$.

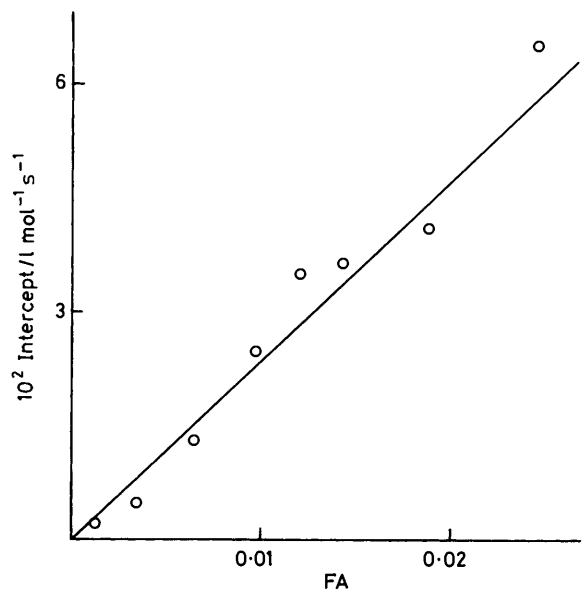


Figure 5. Plot of the intercept *versus* FA for reaction of acetate buffer with ETC as derived from the plot of k_1/total acetate concentration *versus* total acetate buffer concentration. Line is theoretical from parameters in Table 1 and equation (11)

Table 1. Rate constants for formation of intermediate from acetate and ETC catalysed by general acids [a]

Acid	pK_{HA}	k_{HA}/ l^2 mol^{-2} s^{-1}	No.[b]	pH (D)
Oxonium ion	−1.7	8.2 10^4 [d]	39	6.15—7.5
		3.4 10^5 (D)	4 (D)	7.42 (D)
Acetic acid	4.5	3.3	39	6.15—7.5
	5.12 (D)	1.2 (D)	4 (D)	7.42 (D)
NN-Dimethyl-amino-acetonitrile [c]	4.5	3.4	4	6.76
N-Propargyl-morpholine [c]	5.54	0.71	6	6.75
N-Chloroethyl-morpholine [c]	6.27	0.34	11	6.45—7.24
N-Methyl-morpholine [c]	7.8	0.059	6	6.76
	8.4 (D)	0.02 (D)	3 (D)	7.49 (D)
Trimethylamine	10.0	0.0065	4	6.76

[a] 25°, ionic strength maintained at 1M with KCl. [b] Number of data points. [c] HA is the conjugate acid of this amine. [d] The kinetically equivalent second-order rate constant k_{HOAc}[HOAc][ETC] has an isotope effect of 0.80 derived from the above data and the equilibrium isotope effect for acetic acid (K_A^H/K_A^D = 3.33 from S. Korman and V. K. LaMer, *J. Am. Chem. Soc.*, 1936, **58**, 1396; V. K. LaMer and J. P. Chittum, *ibid.*, p. 1642).

The pH dependence of k_1 for buffers containing 1M total acetic acid is illustrated in Figure 2 and the line is theoretical from equation (10); the rate constants are not easily accessible below pH 6 as they are very fast.

The value of k_1 for the reaction of acetate with ETC depends on the acidic form of the general buffer species present. The kinetic rate law of equation (12) is obeyed and is a general form of (10). The third-order parameters (k_{AH})

$$k_1 = \Sigma k_{HA}[HA][AcO^-] \quad (12)$$

deduced for general buffers (Table 1) give considerable scope for kinetic ambiguity but we show later that the present

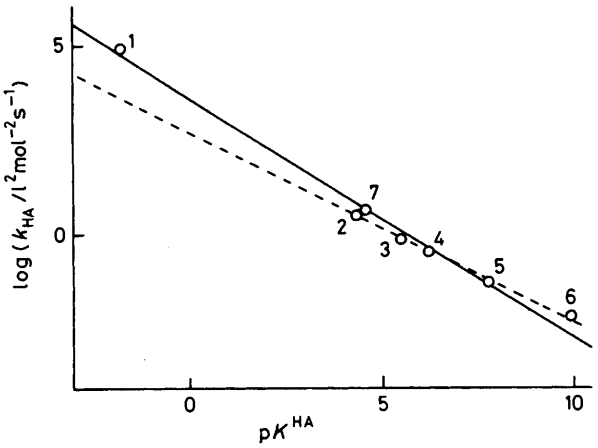


Figure 6. Brønsted relationship for k_{HA} *versus* pK^{HA} for attack of acetate ion on ETC catalysed by HA; the line is theoretical with slope 0.67. Oxonium ion, 1; acetic acid, 2; *N*-propargylmorpholinium ion, 3; *N*-chloroethylmorpholinium ion, 4; *N*-methylmorpholinium ion, 5; trimethylammonium ion, 6; *NN*-dimethylaminoacetonitrile conjugate acid, 7. The dotted line of slope 0.5 correlates the general acids excluding the oxonium ion

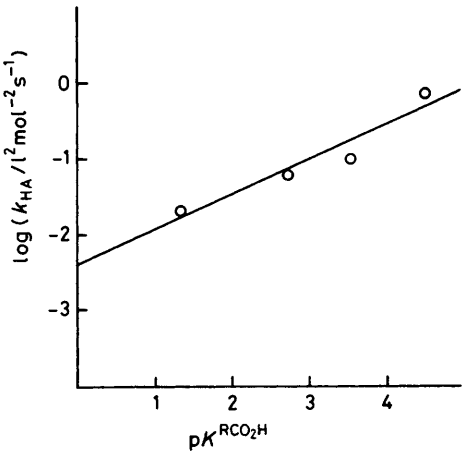


Figure 7. Brønsted relationship between k_{HA} and pK^{RCO_2H} for the reaction between carboxylate anions and ETC catalysed by *N*-chloroethylmorpholinium ion; the line is theoretical with slope 0.46

Table 2. Rate constants for the reaction of carboxylate ions with ETC catalysed by *N*-propargylmorpholine [a]

Carboxylate	pK^{RCO_2H}	10^2k_{HA}/l^2 mol^{-2} s^{-1}	No.[b]	pH
Chloroacetate	2.75	6.1	5	6.7
Dichloroacetate	1.35	2.1	5	6.67
Ethoxyacetate	3.58	10	4	6.7

[a] 25°, ionic strength kept at 1M with KCl. [b] Number of data points.

Table 3. Reaction of 2,2-diethylmalonic acid with ETC [a]

Buffer conc. (M)	10^3k/s^{-1}	10^3k/[Buffer]/ l mol^{-1} s^{-1}	X [b,d]
2,2-Dimethylmalonic acid			
0.4	14.4	36	15.1
0.3	10.3	34	13.8
0.2	7.4	37	12.6
0.1	3.5	35	11.9
N-Chloroethylmorpholine (0.2M-2,2-diethylmalonic acid)			
		10^3Y/s^{-1} [c,d]	
0.2	7.6	5	
0.1	7.2	3.8	
0.05	7.8	3.1	
0	7.4	2.5	

[a] 25°, ionic strength maintained at 1M with KCl. [b] X is the value 10^3k/[Buffer]/l mol^{-1} s^{-1} for replacement of acetate for the malonate at same pH. [c] Y is the value replacing malonate by acetate at the same concentration and pH. [d] See Results section for explanation.

interpretation [equation (12)] is correct. Figure 6 illustrates a Brønsted relationship between k_{HA} and the pK of the acid catalyst.

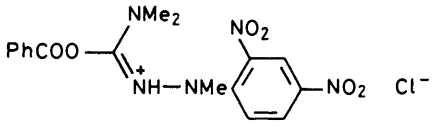
Third-order kinetics are often difficult to justify as they can give rise to only a small percentage of the total reaction flux. The percentage of reaction carried by the term k_{HOAc}[HOAc][AcO$^-$] at 1M total acetic acid buffer and pH 6.86 is *ca.* 60% of the total and this value drops to *ca.* 40% at pH 4.5 as calculated from the parameters in Table 1. These proportions give us considerable confidence that the third-order term exists and the parameters in Table 1 are accurate. The contribution of the third-order term in this reaction is slightly larger than that of trimethylamine oxide and almost double that of acetic acid in the third-order acid base term for the enolisation of acetone (k[$Me_3\overset{+}{N}OH$][$Me_3\overset{+}{N}O$][acetone] and k[AcOH][AcO$^-$][acetone] respectively). We calculate respectively 50 and 27% for 1M total catalyst at a pH corresponding to the pK of the conjugate acid using figures taken from Hegarty and Jencks [10] and from Bell and Jones.[11]

Reaction of ETC with General Carboxylic Acids.—The effect on k_{HA} of changing the carboxylic acid nucleophile while retaining a common general acid (*N*-propargylmorpholine HCl) was measured. The parameter k_{HA} was obtained from the variation of k_1 at constant pH and general acid concentration as a function of carboxylate concentration assuming a rate law of the type of equation (12). The derived data are collected in Table 2 and illustrated in Figure 7.

Reaction of ETC with 2,2-diethylmalonic acid was studied in order to investigate the possibility that this dicarboxylic acid could provide *internal* general acid catalysis. In contrast with the results for acetic acid we observed no curvature in the plot of rate constant *versus* the concentration of diethylmalonic acid where the ratio k_1/[total malonate] is a constant (see Table 3). The result with acetic acid buffer at pH 6.91 indicates that the ratio is not constant (X in Table 3) over the same concentration range employed. Increasing concentrations of *N*-chloroethylmorpholine gave no enhancement whereas for the acetate nucleophile (at the same concentration as the malonate) this amine buffer enhanced the rate constant twofold over the concentration range employed (Y in Table 3). Assuming that the monoanion of 2,2-diethylmalonic acid is reacting with ETC then the second-order rate constant is 8 10^{-2} l mol^{-1} s^{-1} at 25° and 1M ionic strength.

Deuterium Oxide Solvent Isotope Effect.—The solvent isotope effect was measured for the reaction of ETC with acetate catalysed by *N*-methylmorpholinium ion. The 'pH' of the deuterium oxide buffers was recorded and corrected to yield pD by equation (13).[12] The slope of the plot of k_1 *versus* *N*-

$$\text{pD} = \text{pH meter reading} + 0.37 \qquad (13)$$



(3)

methylmorpholinium ion concentration yields the k_{DA} term for attack of acetate ion catalysed by the deuterio-acid (Table 1); the deuterium oxide solvent isotope effect was also measured for the acetic acid–acetate pair (Table 1).

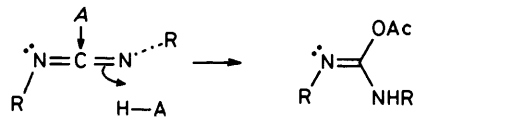
Discussion

Although an *O*-acylisourea intermediate has been postulated for some time as the first stage in the reaction of carboxylic acids with carbodi-imide [1b] there have been few direct demonstrations of its occurrence.[13] *O*-Acylisoureas have been synthesised but from routes alternative to the carbodi-imide method.[14]

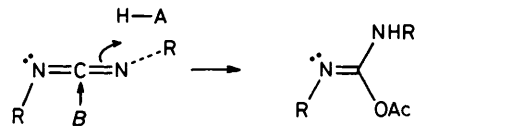
The decomposition of the intermediate from interaction of acetate with ETC is dependent on acetate ion concentration and equation (9) reveals a kinetic ionisation constant (pK 6.8) which is in the region expected for the ionisation of an *O*-acetylisouronium dication intermediate. It is possible to estimate the pK of a model of this intermediate namely PhCO–O–C$^+$(NH$_2$)$_2$ using Charton's equation (pK = −11.01 σ_I + 13.17); [15] the value of σ_I for the benzoyloxy group (0.43) is estimated from the pK of benzoylglycolic acid [16] using Charton's method.[17] The correlation is not very reliable (r 0.815) [15] and the estimated pK (8.43) is judged to possess an error which may encompass the kinetically determined value. The lower observed value may be partly due to the electrostatic effect of the positively charged side chain which could reduce the calculated pK by about one unit. The difference between the pK of propylamine and the lower pK of 1,3-diaminopropane of two units [18] is probably too high as a model system to estimate the electrostatic effect because the amidinium ion has delocalised charge. A better model for the electrostatic effect is the difference in pK values of the carboxy-group in butyric acid and β-alanine (ΔpK *ca.* 1).[18] Pratt and Bruice [19a] find pK values in the range 8—9 for the *S*-benzylisothiouronium salts. Since *S*-methylisothiouronium and *O*-methylisouronium salts have similar pK values (Charton) [15] a monocationic *O*-acetylisouronium species would be expected to have a pK in the 8—9 region confirming the above conclusions. The *O*-benzoylisouronium salt (3) isolated by Hegarty and his co-workers from reaction of silver benzoate with a chloroform-amidine has a pK of 2.36 which is very much lower than the one which we observe. The low value is to be expected from the substituents on the imino function because 2,4-dinitrophenylhydrazine is *ca.* 8 pK units less basic than propylamine.[18]

Decomposition of the fully protonated intermediate is slower than that of Hegarty's [14] in water (1.8×10^{-3} s^{-1} as opposed to 10^{-1} s^{-1} respectively); the higher rate in Hegarty's isourea is probably due to the electron-withdrawing hydrazine system.

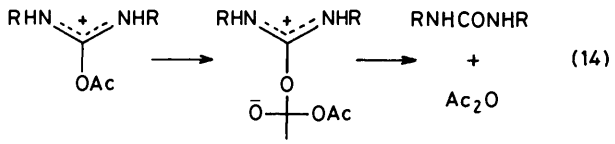
Product analyses indicate that no O ⟶ N acyl group transfer occurs during the reaction from pH 4 to 7. Pratt and Bruice [19] find that the neutral form of *S*-acylisothioureas is the reactive species for the S ⟶ N acyl group transfer. Hegarty and his co-workers [14] observed that the *Z*-form of the acylisourea (3) required specific acid catalysis to convert to the *E*-form which then undergoes rearrangement through the neutral species which now has the correct stereochemistry. It would



(4) *product of attack at A*



(5) *product of attack at B*



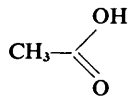
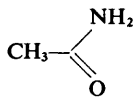
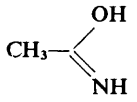
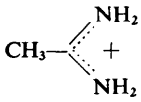
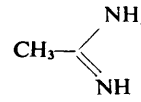
(14)

be expected that carboxylate attack at the carbodi-imide (concerned with proton transfer) would seek the least sterically hindered of the accepting orbitals on the central carbon to yield the *O*-acylisourea (4) with the correct stereochemistry for rearrangement of the neutral species. The absence of *N*-acetylurea in the products of the reaction of ETC with acetate buffers is attributed to the excess of acetate buffer trapping the intermediate as acetic anhydride. In kinetic and product analysis studies the acetate was always the predominant species.

The mechanism for decomposition of the intermediate almost certainly involves reaction of the acetate anion with the cationic isouronium species [equation (14)]; it is envisaged that a zwitterionic tetrahedral intermediate forms which collapses to anhydride and urea. It is very unlikely that the kinetically equivalent mechanism occurs involving attack of acetic acid on the neutral isourea because the proton transfer from acetic acid is thermodynamically favourable at the pH values under question.

The observation of general acid catalysed formation of *O*-acetylisourea is consistent with nucleophilic attack of the acetate on the carbodi-imide concerted with proton transfer to the nitrogen from a donor acid. We consider that the general kinetic rate law [equation (12)] holds where general acid assists nucleophilic attack of the acetate ion because these terms fit a good Brønsted relationship (Figure 6). Rate-limiting proton transfer from the general acid is confirmed by the presence of a normal deuterium oxide solvent isotope effect for the *N*-methylmorpholinium ion and acetic acid (Table 1). The mechanism involving pre-equilibrium protonation followed by rate limiting acetate ion attack [equation (1)] is not consistent with our kinetics as general acid catalysis should not be seen nor should the normal isotope effect. Attack of acetate ion on the carbodi-imide followed by rate-limiting proton transfer from a donor acid [equation (2)] is consistent with the kinetics; the Brønsted plot for this mechanism varying the HA donor should be of the classical ' Eigen ' type.[20] The pK of the *O*-acetylisourea is probably >14 because that for acetamidine is estimated to be *ca.* 19 (Table 4); thus water is likely to be a much better proton donor to the conjugate anion [in equation (2)] than stronger acids on account of a diffusion controlled proton transfer and water's greater concentration. Even with a lower pK for the isourea a zero slope Brønsted relationship should be observed. The kinetically equivalent mechanism for equation (12) namely k[A$^-$]-[HOAc][ETC] may be eliminated because the only function of the conjugate base (A$^-$) would be to remove a proton from

Table 4. Ionisation constants for methyl substituted acids

Acid	pK	Acid	pK
	4.5		17.1 [a]
	7.1 [b]		
	12.52		17.1 + (7.1—4.5) = 19.6

[a] Taken from R. S. Molday and R. G. Kallen, *J. Am. Chem. Soc.*, 1972, **94**, 6739 for *N*-methylacetamide in water. [b] Estimated from the pK of acetamide and the equilibrium constant between acetamide and the isoamide (10^{-8}, A. R. Fersht, *J. Am. Chem. Soc.*, 1971, **9**, 3504.)

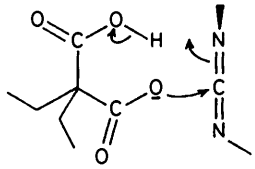
Table 5. Alkaline hydrolysis of 1,3-dicyclohexyl-*O*-phenylisourea [a,b]

[KOH]/M	10^3k/s^{-1}
1	5.11
0.75	3.83
0.5	2.31
0.2	0.93

[a] Ionic strength made up to 1M with KCl, 25°, 30% EtOH–water, kinetics measured at 290 nm. [b] pK of the isourea = 10.60 under the conditions in footnote *a*; k_{OH} = 5.1.10^{-3} l mol^{-1} s^{-1}; pK_w is 14.25 under the conditions of the experiment

acetic acid and at the pH values in question this is not necessary. It is unlikely that the term k_H[H$^+$][AcO$^-$][ETC] should represent the mechanism of equation (4) in its equivalent form k[HOAc][ETC]. Perusal of Figure 6 indicates that the general acids could be correlated by a line which predicts a proton term about an order of magnitude lower than the observed term; there are however no measurements on acids with pK values low enough to give us absolute confidence in such a deviation. The cyclic concerted mechanism would indicate a normal isotope effect whereas a substantial inverse isotope effect (0.8) is seen in the equivalent term k_{HOAc}[HOAc][ETC] derived from the third-order rate constant. We believe that the *proton* term does involve the 'Khorana' pre-protonation mechanism.[1b]

In the present example the third-order term due to proton catalysis (k_H[H$^+$][AcO$^-$][ETC]) constitutes *ca.* 60% of the total reaction flux at pH 4.5 where [AcO$^-$] = [AcOH] = 0.5M. At higher pH values the reaction flux through this path gets less. Thus a considerable amount of the reaction takes the concerted path; it is expected that the carbodi-imides used in peptide synthesis in non-polar solvents will prefer the concerted route because the concentration of protons will be kept very small by the solvent while that of the general acid will be unaffected and thus at relatively high concentration. Moreover the non-polar solvent will not favour the charge separation required for the stepwise mechanism. Dvorko and his coworkers did not observe general acid catalysis in reactions of dicyclohexylcarbodi-imide with carboxylic acids in tetrahydrofuran.[2h] It is possible that the cyclic mechanism [equation (4)] although not seen in this study is acting in Dvorko's case; charge separation as required in the Khorana mechanism [1b] would certainly be repressed. It is not clear from their



(15)

papers whether Dvorko's group should have seen catalysis under the conditions of their experiments.

The reaction of ETC with the monoanion of 2,2-diethylmalonic acid does not require external acid catalysis because intramolecular proton transfer is now possible [equation (15)]. Direct comparison of the rate constants for malonate and acetate reactions is not possible because the nucleophiles and acids have different pK values and hence electronic reactivities. We may calculate the expected differences in reactivity for the acid catalysis using the Brønsted sensitivity (α 0.67) given in Figure 6 and the ΔpK between acetic acid (4.55) and the second pK of 2,2-diethylmalonic acid (7.29). We may also obtain the difference in reactivity of the carboxylate nucleophile from the difference in pK of acetic acid and the first pK of 2,2-diethylmalonic acid (2.21) using the Brønsted type selectivity (β_N 0.46) as in Figure 7. A bifunctional species with nucleophile and acid of pK 4.55 has a predicted bimolecular rate constant given by the calculation of equation (16) and is 54 l mol^{-1} s^{-1}.

$$k_{bimol} = 10^{(7.29-4.55)0.46+(4.55-2.21)0.67} \times 8 \times 10^{-2} \text{ l mol}^{-1} \text{ s}^{-1}$$
$$= 54 \text{ l mol}^{-1} \text{ s}^{-1} \quad (16)$$

Compared with the termolecular term of 3.5 l^2 mol^{-2} s^{-1} for acetic acid this leads to an effective molarity of 15M well within the range expected for the comparison of reactions catalysed inter- and intra-molecularly by proton transfer.[21,22] This value is of course an upper limit depending on the particular value chosen for the Brønsted α value (see earlier).

The observation of concerted general acid catalysis of the addition of carboxylates to carbodi-imide may be rationalised in terms of an energy surface where the intermediates in the stepwise paths [corresponding to equations (1) and (2)] are of high energy thus forcing the reaction co-ordinate to be concerted. The pK of the donor acid lies between the pK values of the two possible intermediates consistent with a concerted mechanism.[23] The reverse reaction, namely the elimination of acetic acid from *O*-acetylisourea to yield the carbodi-imide, will, by the principle of microscopic reversibility, be concerted general base.

The catalysis observed in this system may be classified as a class e process where a general acid assists the attack on the electrophile. Such catalysis is not seen in addition to CO_2, CSO, CS_2, RNCO, and RNCS systems because the anion produced initially is relatively stable; we might expect class e catalysis when the nucleophile becomes very weak. In the present system, more powerful nucleophiles such as phenolate ion react with carbodi-imide without acid catalysis; under these circumstances the energy surface becomes skewed and the anionic intermediate becomes favourable.

If the protonating acid becomes powerful enough, as for example oxonium ion, the protonated carbodi-imide becomes less energetic relative to reactants and the pre-equilibrium protonation mechanism [equation (1)] is preferred.

Class n type catalysis, where a base aids nucleophilic attack by proton removal, is not feasible in the present system. Such catalyses have been observed with other heteracumulenes for example in the reaction between weakly basic alcohols and carbon disulphide to give alkyl xanthates in water [24] and between methanol and phenyl isocyanate in tetrachloroethane.[25a] In the latter case the concerted mechanism prevents

build up of localised charge in a non-polar medium. If in the light of recent evidence carbamoyl group transfer in non-aqueous solvents involves isocyanate formation the observation of base catalysis [25b–d] is consistent with a class e mechanism for the addition to isocyanates.

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References

1 (*a*) Preliminary account, I. T. Ibrahim and A. Williams, *J. Chem. Soc., Chem. Commun.*, 1980, 25; (*b*) M. Smith, J. G. Moffatt, and H. G. Khorana, *J. Am. Chem. Soc.*, 1958, **80**, 6204.
2 D. F. DeTar and R. Silverstein, *J. Am. Chem. Soc.*, 1966, **88**, (*a*) 1013; (*b*) 1020; (*c*) D. F. DeTar, R. Silverstein, and F. F. Rogers, *ibid.*, 1966, **88**, 1024; (*d*) T. A. Budazhhapova, D. G. Knorre, and O. A. Mirgorodskaya, *Izv. Sib. Otd. Akad. Nauk. SSSR, Ser. Khim.* 1967, 73; (*e*) D. G. Knorre and O. A. Mirgorodskaya, *Dokl. Akad. Nauk. SSSR*, 1969, **186**, 340; (*f*) D. F. Mironova, G. F. Dvorko, and T. N. Skuratovskaya, *Ukr. Khim. Zh.*, 1969, **35**, 726; (*g*) D. F. Mironova and G. F. Dvorko, *ibid.*, 1975, **41**, 840; (*h*) D. F. Mironova, G. F. Dvorko, and T. N. Skuratovskaya, *ibid.*, 1970, **36**, 190; (*i*) D. F. Mironova and G. F. Dvorko, *ibid.*, 1967, **33**, 602.
3 I. T. Ibrahim and A. Williams, preceding paper.
4 A. Williams, S. V. Hill, and I. T. Ibrahim, *Anal. Biochem.*, 1981, **114**, 173.
5 A. Williams and I. T. Ibrahim, *J. Am. Chem. Soc.*, 1981, **103**, 7090.
6 J. H. Biel and F. DiPierro, *J. Am. Chem. Soc.*, 1958, **80**, 4609.
7 R. A. Turner, *J. Am. Chem. Soc.*, 1946, **68**, 1607.
8 S. Thea and A. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1981, 72.
9 A. R. Butler and V. Gold, *J. Chem. Soc.*, 1961, 2305.
10 A. F. Hegarty and W. P. Jencks, *J. Am. Chem. Soc.*, 1975, **97**, 7188.
11 R. P. Bell and P. Jones, *J. Chem. Soc.*, 1953, 88.
12 A. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1975, 947.
13 (*a*) A. Arendt and A. M. Kolodziejczyk, *Tetrahedron Lett.*, 1978, 3867; (*b*) G. Doleschall and K. Lempert, *ibid.*, 1963, 1195; (*c*) A. F. Hegarty and T. C. Bruice, *J. Am. Chem. Soc.*, 1970, **92**, 6561, 6569.
14 A. F. Hegarty, M. T. MacCormack, K. Brady, G. Ferguson, and P. J. Roberts, *J. Chem. Soc., Perkin Trans. 2*, 1980, 867.
15 M. Charton, *J. Org. Chem.*, 1965, **30**, 969.
16 C. Concilio and A. Bongini, *Ann. Chim. (Italy)*, 1966, **56**, 417.
17 M. Charton, *J. Org. Chem.*, 1964, **29**, 1222.
18 W. P. Jencks and J. Regenstein in ' Handbook of Biochemistry,' ed. H. A. Sobers, Chemical Rubber Co., Cleveland, 1968, p. J150.
19 R. F. Pratt and T. C. Bruice, *J. Am. Chem. Soc.*, 1972, **94**, 2823.
20 M. Eigen, *Angew. Chem. Int. Ed. Engl.*, 1964, **3**, 1.
21 R. P. Bell and M. A. D. Fluendy, *Trans. Faraday Soc.*, 1963, **59**, 1623.
22 E. T. Harper and M. L. Bender, *J. Am. Chem. Soc.*, 1965, **87**, 5625.
23 W. P. Jencks, *Chem. Rev.*, 1972, **72**, 705.
24 R. J. Millican and C. K. Sauers, *J. Org. Chem.*, 1979, **44**, 1664.
25 (*a*) R. B. Moodie and P. J. Sansom, *J. Chem. Soc., Perkin Trans. 2*, 1981, 664; (*b*) Y. Furuya, K. Itoho, and H. Miyagi, *Bull. Soc. Chem. Jpn.*, 1968, **42**, 2348; (*c*) Y. Furuya, K. Itoho, and S. Fukutaka, *ibid.*, 1970, **43**, 3846; (*d*) Y. Furuya, S. Goto, I. Urasaki, and A. Morita, *Tetrahedron*, 1968, **24**, 2367.
26 M. L. Bender and W. A. Glasson, *J. Am. Chem. Soc.*, 1959, **81**, 1590; J. R. Kirsch and W. P. Jencks, *ibid.*, 1964, **86**, 833.
27 Calculated from M. Kandel and E. H. Cordes, *J. Org. Chem.*, 1967, **32**, 3061.
28 A. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1973, 1244.
29 T. A. Briody, A. F. Hegarty, and F. L. Scott, *Tetrahedron*, 1977, **23**, 1469.
30 R. Stewart and J. P. O'Donnell, *Can. J. Chem.*, 1964, **42**, 1694.

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